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Micro- and macrovascular abnormalities in systemic sclerosis

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CHAPTER 5

DECREASED CAPILLARY PERMEABILITY AND CAPILLARY DENSITY IN PATIENTS WITH SYSTEMIC SCLEROSIS USING LARGE-WINDOW SODIUM FLUORESC EIN VIDEODENSITOMETRY OF THE ANKLE

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ABSTRACT

Objective: Local capillary permeability in patients with systemic sclerosis (SSc) has been reported increased when assessed by nailfold capillaroscopy. We measured capillary permeability at a clinically less affected site by using large-window fluorescein videodensitometry of the ankle. We hypothesized that increased capillary permeability or leakage is a generalized phenomenon in SSc.

Methods: Large-window videodensitometry with sodium fluorescein was performed in 38 SSc patients and 20 healthy controls. Capillary permeability was expressed as the average relative light intensity over the first 7 minutes ($I_{av}(7)$) after appearance of fluorescein in skin capillaries.

Results: Capillary permeability, expressed as $I_{av}(7)$ was significantly decreased in patients with SSc ($47.3 \pm 15.0\%$ vs $57.6 \pm 9.4\%$ in controls, $p=0.007$), as was capillary density ($12 \pm 6 /\text{mm}^2$ vs $26 \pm 11/\text{mm}^2$, $p<0.001$). Adjustment for capillary density in multivariate regression analysis demonstrated that differences in $I_{av}(7)$ between SSc patients and controls were related to differences in capillary density, BMI and HDL cholesterol.

Conclusion: At the level of the ankle decreased capillary permeability was found in SSc patients, related to decreased capillary density. Microvascular involvement in SSc is widespread, but no evidence was established for increased capillary permeability at the level of individual capillaries as a generalized phenomenon.

INTRODUCTION

Systemic sclerosis (SSc) is a systemic autoimmune disease characterized by immunologic abnormalities, fibrosis of the skin and internal organs, and widespread microvascular involvement. Clinical features of vascular involvement include Raynaud's phenomenon (RP), but also major complications like pulmonary hypertension and renal crisis. Vascular or endothelial dysfunction has been suggested to be a crucial element in the pathogenesis of SSc, and increased capillary permeability and vascular leakage is one of its manifestations.¹⁻³

Microvascular abnormalities can be detected by nailfold capillaroscopy. These abnormalities in nailfold capillaries in SSc consist of structural alterations of the capillaries and a reduced capillary density. Bushy patterns, extravasates and giant capillaries are present.⁴⁻⁸ Bollinger et al. found increased capillary permeability in SSc using dynamic fluorescence videomicroscopy of the nailfold.⁹ By means of this approach fluorescein leakage was studied at the single capillary level. It is unknown whether increased capillary permeability in SSc is a local or a generalized phenomenon. Sodium fluorescein (NaF) videodensitometry of the ankle, using a 'large-window' technique, visualizes a larger number of capillaries. Using this technique, we found increased NaF leakage as a marker of increased capillary permeability in patients with diabetes mellitus, and especially in those with microalbuminuria.¹⁰⁻¹² The main advantage of this technique is its reproducibility with a mean of the coefficients of variation over 3 experiments of 10% in diabetic patients and controls.¹⁰ This allows the technique to be feasible and useful for intervention studies.

In this study, we investigated microvascular permeability in SSc patients compared to healthy controls by using large-window videodensitometry with sodium fluorescein at the level of the ankle, in order to test the hypothesis that capillary leakage is a generalized phenomenon in SSc.

METHODS

Subjects

Consecutive patients with SSc according to the ACR criteria¹³ attending the outpatient clinic of our hospital were asked to participate in this study. All patients suffered from RP, confirmed by a previously observed abnormal response to cooling at plethysmography, and all had anatomical capillary abnormalities at nailfold capillary microscopy. A control group of age matched non-smoking healthy subjects was used who had no RP or any other concomitant disease. Exclusion criteria were pregnancy or lactation, smoking, diabetes mellitus, cardiovascular disease, cerebrovascular disease, peripheral vascular disease, renal failure, hepatic failure and autonomic neuropathy. Data were obtained from all subjects with respect to body mass index (BMI) and lipid levels. Ethical approval was obtained from the Medical Ethical Committee of the University Medical Center Groningen, and all participants provided written informed consent.

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Sodium fluorescence videodensitometry

Large-window sodium fluorescein (NaF) videodensitometry was used to measure skin capillary permeability according to the method described by Jager et al.¹⁰ Subjects were studied while lying on their right side. The room temperature was approximately 24 °C. The local skin temperature of the medial ankle was monitored using a thermocouple (Ellab DU3-s, Copenhagen, Denmark) and kept between 28 °C and 32 °C. The system consists of an epiillumination microscope (Olympus BHMJ, Tokyo, Japan) to which a 75 W Xenon lamp (Osram XBO, Berlin, Germany) is mounted. Emitted light is filtered using a fluorescence filter set (Olympus BH2-UDMB, excitation 380-490 nm, barrier 515 nm, Tokyo, Japan). A 2 by 3 mm (6 mm²) section of the skin of the medial malleolus of the ankle was visualized (magnification x100). Immersion oil (Leitz, din 58884, Wetzlar, Germany) was applied to the skin to increase transparency. A bolus of NaF solution (0.3 ml of a 15% NaF solution per litre of estimated blood volume) was injected intravenously. The epiillumination microscope visualizes the rapid capillary appearance and the subsequent interstitial leakage of NaF. Images were recorded by a video camera (Grundig FA-85, Fürth/Bay, Germany), in which an automatic gain function was removed, and a S-VHS video recorder. Images were digitized from tape recording (Data Translation 2862 framegrabber with Iris software) every second for 20 minutes after first appearance of the dye. Dye arrival time (DAT) was defined as the interval from injection of the dye until appearance in skin capillaries.¹² The number of capillaries visualized in the 2 by 3 mm section was counted 60 sec after appearance of the dye from tape recordings. The fluorescence light intensity (FLI) of each image was computed and expressed in arbitrary units. One baseline image was digitized to obtain background FLI, which was subtracted from subsequent intensities. Individual maximum intensity (I_{\max}) was set at 100%. All other intensities were expressed as percentages of I_{\max} . The average relative intensity over the first 7 minutes ($I_{\text{av}}(7)$) after appearance of the dye was used as a parameter of NaF transcapillary and interstitial diffusion. This value showed a day-to-day reproducibility, expressed as coefficients of variation, of 10%.¹⁰

Statistical analysis

Data are expressed as mean \pm standard deviation, when variables were normally distributed. In case of a non-normal distribution data are reported as median and interquartile ranges. Between-group comparisons were performed using parametric or non-parametric tests. Entire relative NaF leakage curves were compared by ANOVA for repeated measurements. Univariate analysis was performed using Spearman's correlation coefficient. Multiple regression analysis was used to assess the relationship between $I_{\text{av}}(7)$, the presence of SSc, and demographic and clinical characteristics. An unadjusted analysis in which no corrections were made for confounders, and an adjusted analysis in which corrections were made for confounders are presented. A variable was defined to be a confounder when significantly changing the regression coefficient B in the model testing the association between patient category (SSc patients and healthy controls) and capillary permeability ($I_{\text{av}}(7)$). Two-tailed p-values <0.05 were considered significant.

RESULTS

SSc patients had a median disease duration of 7 years (IQR 3-12). 90% of patients had limited cutaneous SSc, and 10% diffuse cutaneous SSc. Both subsets were taken together in the analysis because of the low number (4 out of 38) of patients with diffuse cutaneous SSc. Almost half of the patients (47%) had anticentromere autoantibodies, 5% Scl-70 antibodies, 8% no antibodies, and 39% had non-specified antinuclear antibodies. Immunosuppressive agents, such as methotrexate, azathioprine and cyclophosphamide, were used in 32 % of SSc patients during the study, while 16% of patients used corticosteroids. Baseline characteristics of SSc patients and healthy controls are shown in table 1. Significant differences between patients and controls were found in gender, BMI, systolic and diastolic blood pressure, HDL- and LDL-cholesterol. Differences in blood pressure can be explained by the use of vasodilators, such as calcium channel blockers and ketanserin, and other oral medications for the treatment of RP, such as angiotensin converting enzyme (ACE) inhibitors, in SSc patients. Since RP and hypertension were exclusion criteria for healthy controls, use of vasodilating agents was statistically different between groups. No difference was present regarding the use of statins.

Table 1. Characteristics of SSc patients and healthy controls

	SSc patients	Controls	p-value
N	38	20	
Male (n (%))	3 (8%)	7 (35%)	0.023
Age (years)	54.3 ± 11.0	53.2 ± 15.3	0.750
BMI (kg/m ²)	23.7 ± 2.9	27.6 ± 4.4	<0.001
Systolic blood pressure (mm Hg)	120 (110-131)	137 (124-156)	0.004
Diastolic blood pressure (mm Hg)	75 (70-80)	84 (79-93)	0.001
ACE-inhibition therapy (n (%))	6 (16)	0	0.084
Calcium channel blockers (n (%))	22 (58)	0	<0.001
Total cholesterol (mmol/l)	5.39 ± 0.97	6.19 ± 1.81	0.096
HDL-cholesterol (mmol/l)	1.45 (1.23-1.85)	1.07 (0.95-1.37)	0.001
LDL-cholesterol (mmol/l)	3.18 ± 0.79	4.71 ± 1.33	<0.001
Triglycerides (mmol/l)	1.34 (1.05-2.03)	1.15 (0.79-1.87)	0.286
Statins (n (%))	3 (8%)	0	0.544
Skin temperature ankle (°C)	30.2 ± 1.1	30.1 ± 0.7	0.846
Capillary density (n/mm ²)	12 ± 6	26 ± 11	<0.001
Background intensity (AU)	344 (321-851)	716 (511-1102)	0.005
Dye arrival time (s)	46 ± 26	48 ± 14	0.707
NaF leakage I _{av} (7) (%)	47.3 ± 15.0	57.6 ± 9.4	0.007

Data are expressed as mean ± standard deviation, or as median (interquartile range).

SSc, systemic sclerosis; ACE, angiotensin converting enzyme; HDL, high density lipoprotein; LDL, low density lipoprotein; I_{av}(7), average relative fluorescence light intensity over the first 7 minutes

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Capillary density was significantly decreased in patients compared to controls ($12 \pm 6/\text{mm}^2$ vs $26 \pm 11/\text{mm}^2$, $p < 0.001$). Also background fluorescence was decreased in SSc patients. Dye arrival time (DAT) was not different between groups. Transcapillary leakage of NaF, expressed as the average relative intensity over the first 7 minutes after arrival of the dye ($I_{av}(7)$), was significantly lower in SSc patients ($47.3 \pm 15.0\%$ vs $57.6 \pm 9.4\%$ in controls, $p = 0.007$), supporting reduced capillary permeability (Table 1). The relative fluorescence intensity curve was also significantly lower in SSc patients than in healthy controls ($p = 0.006$, figure 1). Univariate analysis performed using data of all subjects showed only a significant positive correlation between $I_{av}(7)$ and capillary density and BMI. Multivariate analysis disclosed that differences in $I_{av}(7)$ between SSc patients and healthy controls were related to differences in capillary density, BMI and HDL cholesterol (table 2). Addition of other confounders, that is diastolic tension, triglycerides, use of calcium channel antagonists or angiotensin converting enzyme inhibitors, and gender, did not change the outcome.

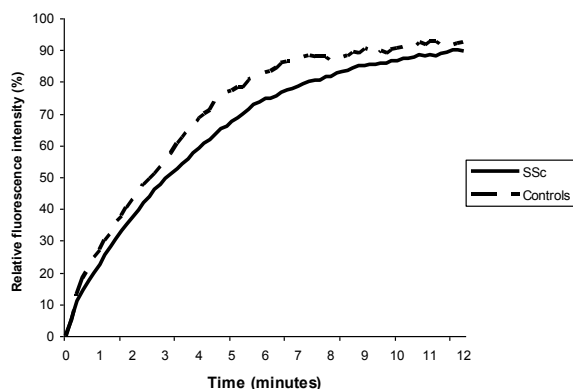


Figure 1. Relative fluorescence light intensity (%) after NaF arrival in the skin of SSc patients (—) and healthy controls (---).

Table 2. Linear regression analysis of determinants of NaF leakage ($I_{av}(7)$) between SSc patients and healthy controls

Group (patients, controls)	B [#]	95% confidence interval for B	p-value
Unadjusted/crude	10.326	2.943; 17.708	0.007
Adjusted*	-12.451	-25.921; 1.018	0.069

*In the adjusted model corrections were made for the confounders capillary density/ mm^2 , BMI and HDL cholesterol; [#]B is the regression coefficient

DISCUSSION

In the present study, we evaluated capillary permeability in SSc patients at a clinically less affected site by using large-window fluorescein videodensitometry of the ankle in order to test the hypothesis that capillary leakage is a generalized phenomenon in SSc. Unexpectedly, we found a significantly impaired NaF leakage in SSc patients, but this impaired NaF leakage could be explained by a decreased capillary density at the level of the ankle in SSc patients. Compared to SSc patients, higher levels of NaF leakage in healthy controls could be explained by higher BMI and lower HDL cholesterol levels, indicating a more unfavourable cardiovascular risk profile.

In patients with SSc, microangiopathy with increased capillary permeability of the nailfolds is characterized by loss of capillaries, morphologic changes in the capillaries, asymmetric leakage of the dye, partial or complete loss of dye concentration at the halo (i.e., outer border of the skin papilla), and preferential accumulation sites of dye in the remote interstitial space.⁹ Most prominent abnormalities in patients with SSc are present in the nailfolds. Probably, the frequent episodes of digital ischemia in SSc patients with often severe Raynaud's phenomenon may be responsible for the reported increase in local nailfold capillary permeability. We found an impaired capillary permeability at the level of the ankle, but also a decreased capillary density. The decrease in capillary density might suggest that microvascular involvement is not restricted to the nailfolds in SSc patients. Limited data is available about capillary density elsewhere in the body in SSc patients. Using labial capillary microscopy, Grassi et al.¹⁴ were not able to find decreased capillary density in SSc patients compared to controls, even in patients with definite avascular areas in the nailfold, whereas patients did show widespread disorganisation of the capillaries. However, evaluating muscle biopsies from SSc patients, Scarpelli et al demonstrated a decreased capillary density compared to patients with polymyositis, rheumatoid arthritis, muscle dystrophy and neurogenic atrophy.¹⁵ In the present study, dye arrival time was similar in patients and controls, suggesting no differences in total skin flow. Since NaF leakage was reduced as was capillary density, we cannot exclude that permeability at a single capillary level was increased, possibly in order to compensate for the decreased capillary density. However, we cannot confirm our hypothesis that increased capillary permeability is a generalized phenomenon.

Since Raynaud's phenomenon is present in more than 95% of SSc patients and microvascular abnormalities, such as a decrease in capillary density, are present in SSc, increased capillary permeability was hypothesized by Grassi et al. to be a protective mechanism at least in the digits. Increased permeability has been supposed to allow enhanced nutritional exchange in the remaining capillary loops, but this hypothesis has not been substantiated further.¹⁶ Cold exposure has been shown to decrease capillary flow, resulting in a significant increase in dye arrival time and pericapillary and interstitial fluorescent light intensities.¹⁷ These cold-induced changes in fluorescent light intensities were counteracted by the use of nifedipine in SSc patients.¹⁸ In the present study, use of vasodilating agents, such as calcium channel blockers and ACE inhibitors, might have influenced NaF leakage, since these agents were significantly more frequently used in SSc patients. ACE-inhibitors are known to reduce NaF leakage, but their use, and also the use of calcium channel blockers, were not correlated with NaF leakage in this study (data not shown).¹²

Although controls with cardiovascular disease and other known risk factors possibly influencing capillary permeability were excluded, significant differences between patients and controls were present in, amongst others, BMI and lipid levels. BMI and HDL cholesterol were confounders in the association between capillary permeability and patient category (SSc patients or healthy controls). Higher levels of capillary permeability in controls compared to SSc patients could also be explained by a more unfavourable cardiovascular risk profile, i.e. higher BMI values and lower HDL cholesterol levels. Low HDL-cholesterol concentration^{19;20} and increased weight^{21;22} are known to be related with endothelial dysfunction.

The decreased background intensity in patients with SSc was unexpected. In patients with diabetes increased background intensity has been observed, probably related to accumulation of advanced glycosylation end-products (AGEs).¹² Skin autofluorescence, related to the accumulation of AGEs, is strongly associated with progression of coronary heart disease and mortality in patients with diabetes, or those in hemodialysis.²³ Using ELISA, accumulation of Nε-(carboxymethyl)lysine, one of the AGEs, was found increased in SSc, and increased levels were associated with early changes at nailfold capillaroscopy, suggesting involvement of AGEs in SSc.²⁴ Therefore, we expected to find increased instead of decreased background intensity. AGE-associated fluorescence rises with age and can also be increased in nondiabetic smokers and in various manifestations of cardiovascular disease.^{25;26} Because AGE-accumulation and skin autofluorescence are strongly related to collagen-linked autofluorescence, and thereby to the half-life of collagen, the previously reported increases in both collagen synthesis and, especially, increased degradation of skin collagen in SSc suggest that reduced skin autofluorescence in our patients may be related to accelerated skin collagen turnover.²⁷⁻²⁹

In conclusion, our study confirms the presence of more generalized microvascular involvement in SSc as capillary density at the level of the ankle was decreased. The decreased capillary permeability in SSc patients could be explained by the decreased capillary density. Moreover, a more unfavourable cardiovascular risk profile, i.e. increased BMI and lower HDL cholesterol levels, were found in controls. This might also explain higher levels of capillary permeability in controls compared to SSc patients. Using large-window videodensitometry, we can, however, not exclude that increased capillary permeability is present at the level of a single capillary to meet the demands of nutritional exchanges in response to reduced capillary density at the level of the ankle. Therefore, we cannot confirm our hypothesis that increased capillary permeability is a generalized phenomenon in SSc.

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